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Denitrification and N mineralization from hairy vetch (*Vicia villosa* Roth) and rye (*Secale cereale* L.) cover crop monocultures and bicultures

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Abstract

N mineralization, N immobilization and denitrification were determined for vetch, rye and rye-vetch cover crops using large packed soil cores. Plants were grown to maturity from seed in cores. Cores were periodically leached, allowing for quantification of NO_3^- and NH_4^+ production, and denitrification incubations were conducted before and after cover crop kill. Gas permeable tubing was buried at two depths in cores allowing for quantification of N_2O in the soil profile. Cover crops assimilated most soil N prior to kill. After kill, relative rates of N mineralization were vetch > rye-vetch mixture > fallow > rye. After correcting for N mineralization from fallow cores, net N mineralization was observed in vetch and rye-vetch cores, while net N immobilization was observed in rye cores. Denitrification incubations were conducted 5, 15 and 55 days after kill, with adjustment of cores to 75% water filled pore space (WFPS). The highest denitrification was observed in vetch cores 5 days after kill, when soil NO_3^- and respiration rates were high. Substantially lower denitrification was observed on subsequent measurement dates and in other treatments probably due to either limited NO_3^- or organic carbon in the soil. On day 5, 3%, 23%, 31% and 31% of the N_2O was recovered in the headspace of fallow, vetch, rye and rye-vetch cores, respectively. The rest was stored in the soil profile. In a field study using intact soil cores, denitrification rates also peaked 1 week after cover crop kill and decreased significantly thereafter. Results suggest greater potential N losses from vetch than rye or rye-vetch cover crops due to rapid N-mineralization in conjunction with denitrification and potential leaching, prior to significant crop N-assimilation.

Introduction

The use of winter annual cover crops has increased in recent years due to their ability to significantly reduce NO_3^- leaching during the winter and spring (Meisinger et al., 1991) and/or provide for the nitrogen (N) demands for subsequent crop growth (Hargrove, 1986; McVay et al., 1989; Power et al., 1991). Unfortunately, no single cover crop has been shown to consistently achieve both objectives. Grasses, for example, can significantly reduce N leaching, but generally provide little N for crop growth (Ebelhar et al., 1984; Hargrove, 1986). Legumes, such as hairy vetch, can supply substantial amounts of N, but their ability to reduce N leaching during the winter and spring is minimal (Meisinger et al., 1990; Ranells and Wagger 1997; Shipley et al., 1992). Based on the above evidence, grass/legume mixtures could have the potential to fulfill both objectives.

Rye-vetch mixtures (seeding ratio 2:1 rye:vetch) are capable of producing greater amounts of foliar biomass and N content than monocultures of either vetch or rye (Clark et al., 1997; Ranells and Wagger, 1996). In a 2-year study, rye plant N concentrations in the mixture ranged from 20 to 100% greater than in the monoculture, presumably due to transfer of N from vetch to rye (Ranells and Wagger, 1996). Yet, maize yields following rye-vetch mixtures tended to be intermediate with vetch > rye-vetch > rye (Clark et al., 1994, 1997).

To assess the ability of cover crop production systems to conserve and better utilize N for crop production, better understanding of the dynamic and fate of N in these systems is needed. While the effects of monocropped cover crop C:N ratios on decomposition has been documented (Ranells and Wagger, 1996; Varco et al., 1993), little information is available regarding the relative potential for N mineralization/immobilization by mixed cover crops or their potential to minimize loss of N via denitrification. Under moderate pH and temperature conditions, the potential for denitrification in soil is regulated by: 1) soil O_2content (which is a function of soil moisture (influencing rate of O_2 diffusion) and biological activity (influencing rate of O_2 consumption)], 2) availability of electrons (e.g. carbon) for NO_3^- reduction and 3) NO_3^- concentration (Firestone, 1982). Cover crop residues have the potential to enhance denitrification, particularly during of periods of soil saturation (i.e. lows rates of O_2 diffusion), because carbon and NO_3^- are readily available. This pathway can lead to substantial soil N losses (Shelton et al., 2000), as well as cause atmospheric release of N_2O which is a potent greenhouse gas. Previous studies have shown that hairy vetch residues can dramatically stimulate soil denitrification rates (Aulakh et al., 1991a, b; McCarty et al., 1999; Shelton et al., 2000). Surface-applied wheat straw residues(C:N =82), however, decreased denitrification rates compared to the control (no residue) (Aulakh et al., 1991a). Little information is available on the denitrification potential of grass/legume mixtures. The objectives of this study were to evaluate the denitrification, potential N leaching, and N sequestration potential of a hairy vetch, rye and rye (50%)/vetch (50%) mixture soon after cover crop kill.

Materials and methods

Experiment 1 – Packed soil cores

The soil in this study was collected from a maize field at Beltsville Agricultural Research Center (Beltsville, MD). The soil was a coarse-loamy Typic Hapludult with an organic C content of 11 mg g^{-1} soil and total N content of 1.2 mg g^{-1}, textural analysis of 62% sand, 18% silt, and 20% clay, and pH of 6.8.

Plant establishment in packed soil cores

The packed-core design, equipment and methodology used in this experiment have been described by Shelton et al. (1996) with recent modifications by McCarty et al. (1999). The soil columns were constructed from aluminum sheeting inserted into Buchner funnels (dimensions of soil column: diameter=16 cm, height=30 cm). A Plexiglas top was fitted with a rubber o-ring to seal the top of each column to allow for headspace sampling (see McCarty et al., 1999 for a diagram of the cores). Soil (6 kg dry weight total) was added to form these columns in three equal increments. After the first and second increments, a coil of gas permeable silicone tubing was placed on each soil layer resulting in coils buried in completed soil cores at about 12 cm and 24 cm below surface, respectively. The ends of the tubing for each of the coils were connected to separate sampling ports fitted in the Plexiglas top. This allowed sampling of gases in the soil pore space, as well as on the soil surface (headspace). Three cores were then planted with hairy vetch (*Vicia villosia*) (20 seeds per core), three with cereal rye (*Secale cereale*) (20 seeds per core), three with a hairy vetch/cereal rye mixture (10 vetch and 10 rye seeds), and 3 were left fallow. The plants were grown in a growth chamber (Controlled Environments Incorporated, Pembina, North Dakota) at 20 °C day (9 h) and 10 °C night and watered weekly on a rainfall simulator (for details see Isensee 1992) for approximately 2 h (2.5 cm h^{-1}) and vacuum applied (0.15 kPa) for 12–15 h. Vacuum was applied to expedite soil water collection. On day 15 and day 31, 100 mg N as KNO_3 was added to all cores to ensure adequate growth of the cereal rye. Total leachate was measured and aliquots were taken and refrigerated for later NO_3^- and NH_4^+ analysis. Content of NO_3^- and NH_4^+ in the leachate were determined colorimetrically by flow injection analysis (Lachat Instruments, Milwaukee, WI). Since the leachate was collected under vacuum, the NO_3^- and NH_4^+ in the leachate represent the potential mineral N leached. Plants were killed with paraquat (4,4′-Bipyridinium, 1,1′-dimethyl-, dichloride) 73 days after planting. The above-ground portions of the plants were then clipped, dried at 70 °C and weighed. Foliage biomass at the time of kill averaged between 9.0 and 9.8 g for all cover crops. Biomass applied to the cores was adjusted slightly such that 9.0 g vetch, 9.0 g rye, and 4.5 g vetch and 4.5 g rye (in a mixture) were placed on the soil surface. This resulted in equal amounts of above-ground residue biomass for each cover crop treatment, however, root biomass may have varied somewhat between treatments.

Table 1. Time course of events in the packed-core experiment

Day(s)	Treatment
1	Planting
	Three experimental cores planted with vetch; three cores planted with rye; three cores planted with a rye-vetch mixture and three cores fallow
14–70 (weekly)	Rain: rain for 2 h (2.5 cm h^{-1}). Vacuum for 12 h. Leachate collected and analyzed for NO_3^- and NH_4^+
14,31	N Fertilization
58	Denitrification Incubation
73	Harvest/Kill; determination of shoot biomass from vetch, rye and rye-vetch cores
74–130 (weekly)	Rain
78,88,138	Denitrification Incubation
Cores incubated at 75% water filled pore space	

Denitrification incubations

Denitrification incubations were conducted in cores with living plants on day 58 after planting and after kill with plant residues on days 78, 91 and 129 (Table 1.). Prior to the denitrification incubations, soil cores were weighed and water added to obtain a 75% water filled pore space (WFPS). Water-filled pore space was calculated as follows: WSPS = (((gravimetric water content × soil bulk density)/total soil porosity) × 100) where soil porosity = (1-(soil bulk density/2.65)) with 2.65 being the assumed particle density of soil. The denitrification incubation has been described previously (McCarty et al., 1998). Briefly, cores were sealed, 200 mL of acetylene added (∼10% concentration), and the headspace gasses re-circulated using a diaphragm pump (1.0 L min^{-1} for 15 min). Cores were sampled (headspace and buried tubing) at 12, 24, 36 and 48 h. Oxygen was added as needed to ensure adequate O_2concentrations in the headspace. Immediately following each denitrification incubation sampling, the diffusion tubing was flushed with 20 mL of He gas and the sampling ports were then sealed from the outside air.

The 2 mL gas samples were analyzed for N_2O, CO_2, O_2 and C_2H_2 (acetylene) via a gas chromatograph (Model 540 GC; Tremetrics Inc., Austin, TX) equipped with a Porapak Q and molecular sieve columns and an ultrasonic detector, interfaced with a headspace autosampler (Model 7000, Tekmar Co., Cincinnati, OH). The volume of the headspace, air-filled pore space and water filled pore space (based on 75% water filled pore space) were 2.37 L, 0.53 L and 1.58 L, respectively. Total N_2O–N (mg) per core was calculated by multiplying the N_2O concentrations in the tubing or headspace by the appropriate volume. Nitrous oxide concentrations in the water-filled pore space, however, were first multiplied by the Oswald coefficient (0.61 at 22 °C; Weiss and Price, 1980) to adjust for N_2O solubility in water at different temperatures and then multiplied by the volume of the water-filled pore space. This procedure has been previously described (McCarty and Blicher-Mathiesen, 1996).

The experimental design was a completely randomized design with three replicate cores per treatment. Analysis of variance was performed with PROC GLM and means separated by LSD (SAS Version 6.12, SAS Institute, Cary, NC). When required, gas production data were log transformed before statistical analysis to equalize variance.

Experiment 2 – Intact soil cores

Study site and sampling collection

The study site was located in Eastern Shore region of Maryland at the Poplar Hill Research Farm (University of Maryland). The soil is a Mattapex silt loam (Aquic Hapludult) with a textural analysis of 19% sand, 67% silt and 14% clay. Replicate field plots were seeded on September 10, 1997 with hairy vetch, rye or were fallow. Plots were fertilized with 160 kg of N ha^{-1} (surface broadcast NH_4NO_3) and cover crops simultaneously killed with paraquat on May 21, 1998 and maize planted on June 3, 1998.

The field design was a randomized block with three replicate plots per treatment. Four cores each were ob-

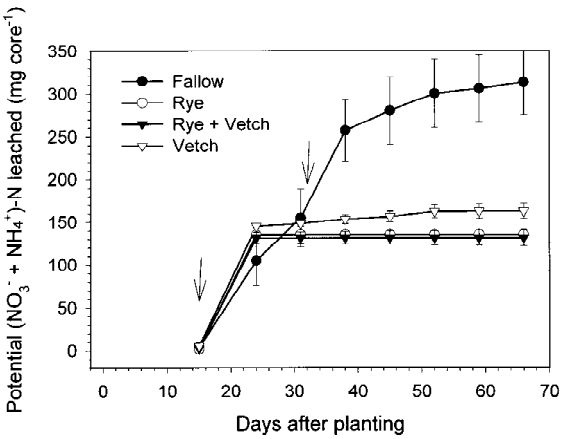

Figure 1. Cumulative inorganic N leached from soil cores after planting cover crop treatments. The arrows indicate the times when external NO_3^- was added to treatments (± standard error). Error bars not seen are contained within the symbol.

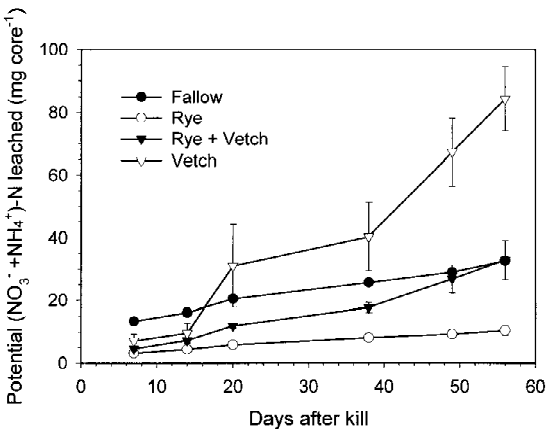

Figure 2. Cumulative N leached from soil cores after cover crop kill (± standard error).). Error bars not seen are contained within the symbol.

tained from two replicate plots of the vetch, rye and fallow treatments. Soil cores were obtained by pounding a steel coring tube (4-cm diameter) containing a plastic cylinder insert into the ground to a depth of 16 cm. The plastic insert containing the intact soil core was then removed and sealed at both ends with stoppers. The plots were sampled 7, 14 and 49 days after planting.

Denitrification and CO_2 production rate measurements

Denitrification rates in intact cores were estimated using the C_2H_2 block technique as described by Parkin (Parkin, 1987; Parkin and Robinson, 1989). Denitrification and CO_2 production rate measurements began immediately upon returning to the laboratory. The gas pressure in cores was brought to atmospheric levels by venting with a needle. Ten ml of C_2H_2 (10% concentration) was subsequently added to each core and the headspace gases mixed by alternatively drawing and releasing a vacuum on the cores using a 60-ml syringe. Following mixing, the gas overpressure was vented. Cores were incubated at 23 °C and gas samples withdrawn for analysis after 8 h. Headspace samples of cores were sampled and analyzed before incubation and any nitrous oxide detected was subtracted from the final value.

Analysis of variance was performed with PROC GLM and means separated by LSD (SAS Version 6.12, SAS Institute, Cary, NC). Gas production data for intact cores were log transformed before statistical analysis to equalize variance.

Results and discussion

Prior to kill, cover crops reduced NO_3^--N and NH_4^+-N leached by approximately one half compared to fallow cores (Figure 1). Among the cover crops, significantly less N ($p < 0.05$) was leached from the rye and the rye-vetch mixture (120 mg N) compared to the vetch (150 mg N). Note that nitrogen conservation by vetch in soil cores may not accurately reflect actual field conditions, because rye plants grow at lower soil temperatures (Duke, 1981) and have more extensive root systems (Meisinger et al., 1991). In field studies using ^{15}N, percent recovery of ^{15}N for rye was 45% compared to only 10% for hairy vetch (Shipley et al., 1992). Thus, a rye cover crop will typically take up more soil N than vetch, when grown in the field.

After kill, substantially more NO_3^-–N and NH_4^+–N were leached from vetch cores than rye, rye-vetch mixture or fallow cores (Figure 2). Cumulative NO_3^- + NH_4^+ losses (after 55 days) were 84, 33, 33 and 10 mg N $core^{-1}$ for the vetch, fallow, rye-vetch and rye treatments, respectively. Ammonium and NO_3^-–N leached in the rye and rye-vetch mixture were equal to or below those in the fallow treatment due to N immobilization in the rye litter. As a general rule, net N mineralization is expected when C:N ratios are < 20, while net immobilization is expected when C:N ratios > 20 (Jenkinson, 1981). C:N ratios for vetch, rye and vetch + rye tissues were 10.3, 21.4 and 14.8, respectively. Consequently, vetch decomposition resulted in net mineralization while rye decomposition resulted in net immobilization. After correcting for N mineralization from fallow cores, net N mineralization from

Table 2. Estimated N mineralization rates for different cover crop components

Treatment	Net N mineralization	Calculated net mineralization for cover crop components[a]
	mg (NO_3^- + NH_4^+)-N d^{-1}	
Fallow	0.53	–
Rye	0.19	−0.34
Vetch	1.51	0.98
Rye + vetch	0.63	0.10

[a] Estimated by subtracting mineralization rate of soil N (fallow treatment). Negative values indicate N immobilization.

vetch residue was 0.98 mg (NO_3^-+ NH_4^+)-N d^{-1} and net N immobilization in rye cores was 0.34 mg (NO_3^- + NH_4^+)-N d^{-1} (Table 2). For the vetch-rye mixture, the observed net N mineralization rate was 0.10 mg (NO_3^- + NH_4^+)-N d^{-1} after correcting for N mineralization from fallow cores. With more extensive experimentation, it may be possible to predict net mineralization rates based on the C:N ratios for different percentages of vetch and rye.

Denitrification incubations

Denitrification, NO_3^- leached, and CO_2 production for the three cover crops and the fallow treatments are shown in Figure 3. Total N losses from denitrification were greatest with hairy vetch residues 5 days after kill (10.7 mg N evolved). Denitrification N losses were substantially lower on days 15 and 55 regardless of the treatment. No statistically significant differences ($p<0.05$) existed between the treatments on day 15 and 55; although there was a trend for greater denitrification N losses in the vetch and rye-vetch cores on day 55 ($p<0.1$).

Little NO_3^- was leached from the cover crop treatments 5 and 15 days after kill; substantially more NO_3^- was leached on day 55, except with rye (Figure 3b). The opposite trend was observed in fallow cores where maximum NO_3^- leaching occurred on day 5. Five days after kill, greater than 90% of the soil NO_3^-–N pool in vetch cores was denitrified, based on denitrification N losses of ca. 11 mg N and NO_3^-–N losses of 1 mg N. On day 55, less than 15% of the total soil NO_3^-–N pool in vetch cores was denitrified, based on denitrification N losses of ca. 2 mg N and NO_3^--N losses of 15 mg N. These data indicate that denitrification was probably initially limited by NO_3^- availability, but that other factor(s) subsequently limited denitrification. Note that under field conditions, NO_3^- losses would be less due to crop assimilation. Respiration rates were consistently high across all cover crop treatments on day 5 and substantially higher than in fallow cores (Figure 3c). Respiration rates decreased pro-

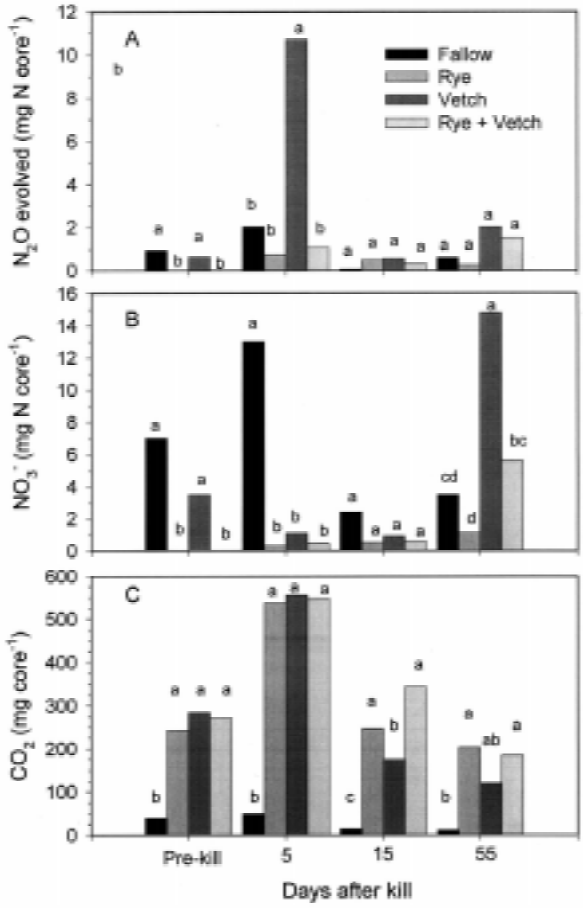

Figure 3. Denitrification assays of packed soil cores: (A) N_2O produced after 48 h in presence of C_2H_2; (B) NO_3^- in leachate after assay; and (C) CO_2 production during 48 h assay.

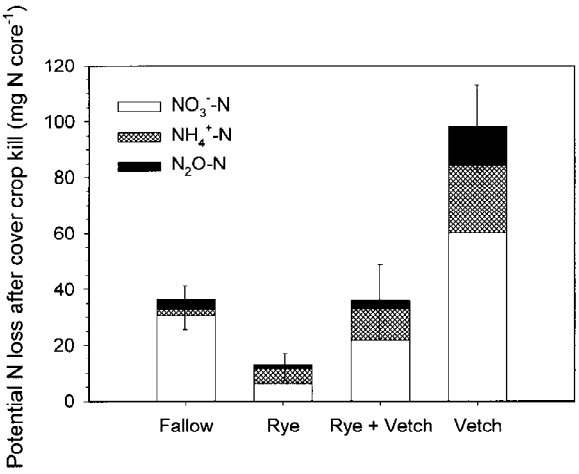

Figure 4. Cumulative N loss via leaching and denitrification after cover crop kill. On the total inorganic N lost from cores, 11%, 12%, 9% and 17% was denitrified from the fallow, rye, rye + vetch, and vetch treatments, respectively.

gressively with time with all cover crops, although rates in vetch cores decreased somewhat more rapidly.

Collectively, these data illustrate the interactive effects of NO_3^- availability and respiration rates (a measure of soil carbon availability) on denitrification. Five days after kill, high denitrification rates were observed in vetch cores likely a result of both high NO_3^- availability (rapid N mineralization) and high organic carbon availability (as seen by high respiration rates). Substantially less denitrification was observed in fallow cores likely due to lower soil organic carbon (low soil respiration) and in rye and rye-vetch cores due to limited NO_3^- availability (slow N-mineralization). Fifteen days after kill, little denitrification was observed in all cores due to limited NO_3^- availability. This was unexpected for the vetch cores. Note, however, that acetylene is a potent inhibitor of nitrification and probably limited NO_3^- production. Ammonium levels leached from vetch and rye-vetch cores was considerably higher than expected, indicative of nitrification inhibition (Figure 4). On day 55, somewhat elevated denitrification was observed in vetch and rye-vetch cores probably due to moderately high respiration rates coupled with NO_3^- availability.

Significantly more N was lost from the vetch than the other treatments (Figure 4). Total inorganic N lost from the vetch cores was 98 mg N, of which 17% was denitrified. Almost half of these losses occurred within the first 30 days after cover crop kill. This rapid release of N may not be ideal for maize growth because it likely precedes the main N demand period. The main N demand period for maize, for example, is 5–6 weeks after planting (Hanway, 1963), or about 7–8 weeks after cover crop kill. Thus, this release of N early in the season by the vetch crop is susceptible to leaching. In a ^{15}N labeled cover crop experiment, consistently more ^{15}N was recovered in a maize crop following a biculture of rye-crimson clover than following a crimson clover monoculture (Ranells and Wagger, 1997).

Denitrification percentages were lower in the other treatments compared with the vetch treatment (Figure 4), however, these represent significant losses of N for crop growth. Nitrogen losses due to denitrification would likely have been substantially greater if the WFPS was increased. Previous studies (Aukulh et al., 1991 b; McCarty et al., 1999; Shelton et al., 2000) suggest a threshold WFPS value of ca. 60% for denitrification; furthermore a 10% increase in WFPS can result in an order of magnitude increase in denitrification (Shelton et al., 2000). Denitrification accounted for between 60 and 70% of the total inorganic N ($NO_3^- + NH_4^+ + N_2O$) when WFPS ranged between 90 and 100% (Shelton et al., 2000).

Consistent with previous studies (McCarty et al., 1999; Shelton et al., 2000), N_2O production rates measured in the headspace (Figure 5) were not representative of overall denitrification. Substantial amounts of N_2O remained trapped in the soil matrix. The percentage of total N_2O–N in core headspaces vs. the soil pores spaces on Day 5 was 3, 23, 31 and 31% in fallow, vetch rye and rye-vetch cores, respectively. In general, gradients of N_2O concentration were observed within the soil profile and between soil and headspace. The most dramatic concentration difference was observed within the profile of vetch cores, where final N_2O concentrations were almost four times greater in the bottom than in the tops of the cores. This illustrates two impacts of gaseous diffusion on measured denitrification: (1) greater denitrification occurred in the lower profile due to longer diffusion pathways of O_2 into the cores and (2) slow diffusion of N_2O out of cores resulted in a N_2O gradient with concentrations increasing with soil depth.

Intact soil cores

Denitrification rates in vetch and rye plots were highest 7 days after cover crop kill, then progressively decreased (Figure 6a). Denitrification rates in vetch and rye plots were not significantly different seven days after kill; on day 14, denitrification rates in vetch plots were significantly higher ($p<0.05$); there were

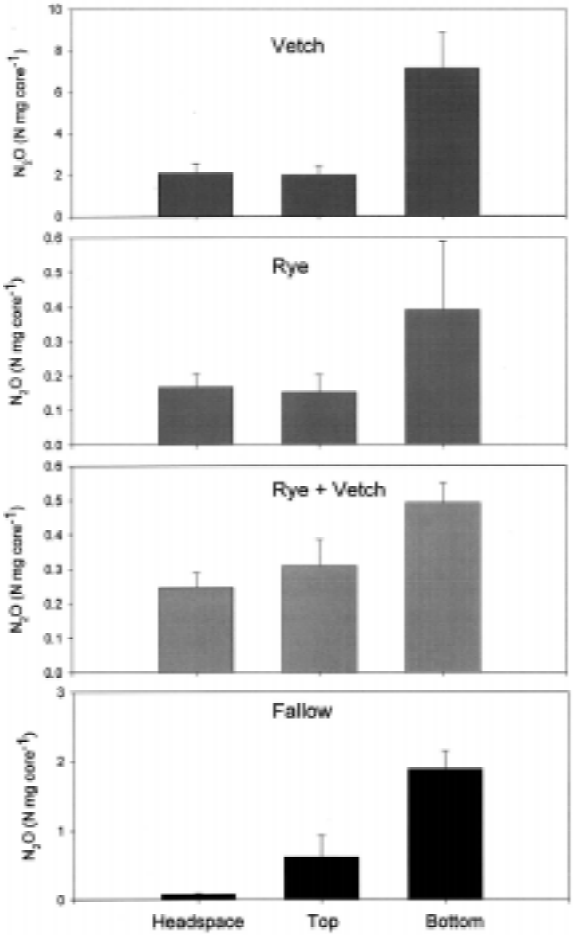

Figure 5. Content of N_2O in headspace and within the soil matrix during denitrification assays (± standard error).

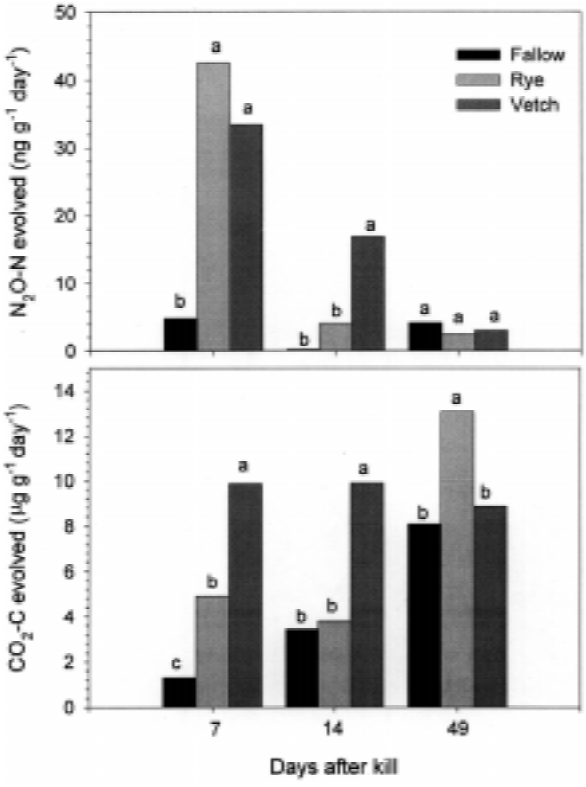

Figure 6. Assay of intact soil cores from the field experiment: Rates of N_2O produced by denitrification in presence of C_2H_2 and CO_2 produced by respiration.

no differences after 49 days. Note that all field plots received 160 N kg ha^{-1} 2 days after cover crop kill; NO_3^-–N concentrations were > 50 μg g^{-1} soil in all treatments on day 7 (data not shown). Consequently, N mineralization rates from cover crops were not a controlling factor for denitrification. These data indicate that the rye cover crop can produce denitrification rates comparable to vetch when NO_3^- is not limiting. Denitrification rates were uniformly low on day 49 likely due to NO_3^- limitation; NO_3^-–N concentrations were < 1 μg g^{-1} soil in all treatments on day 49 (data not shown). Maize silking had commenced, and substantial amounts of N had already been taken up out of the soil by the growing maize plants.

Seven and 14 days after kill, respiration rates were vetch > rye > fallow, although rye and fallow treatments were comparable on day 14. Vetch biomass produces a dense mat of vegetation on the soil surface after kill, while rye stalks remain standing in place; consequently, rye shoot tissue did not contribute to soil respiration. On day 49, relatively high respiration rates were observed in all plots, presumably due to severed maize roots in the intact soil cores.

Comparisons between packed and intact soil cores

Results from the packed and intact soil cores experiments indicated that the highest denitrification rates occurred soon after cover crop kill when both nitrate and soil carbon were readily available. By using legume-grass mixtures, which release N slowly over time, and by delaying N fertilization, denitrification losses should be reduced. Obtaining a representative measure of denitrification in the field is difficult because of inherent soil variability and artifacts arising from the sampling procedure. Neither the packed core nor intact soil core methods provide a complete or unbiased estimate of denitrification losses. Major advantages of the intact core method are: (i) denitrification rates can be determined under actual field conditions,

(ii) the effect of different agronomic practices (e.g. tillage vs. no-tillage) can be directly compared, and (iii) replication allows for characterization of field spatial variability. Limitations include: (i) disruption of soil structure and potential inhibition of denitrification due to oxygen intrusion, (ii) inability to account for N_2O stored in the soil matrix and to determine N mass balances, and (iii) logistical constraints on characterizing denitrification temporal variability. Major advantages of the packed core method are: (i) ability to quantify N_2O, CO_2 and O_2 concentrations in the soil profile (via gas-permeable tubing), (ii) elucidation of temporal variability of denitrification rates as a function of WFPS, and (iii) ability to determine N mass balances for individual denitrification incubations or for a simulated growing season. Limitations include: (i) inability to simulate actual field conditions (e.g. no-tillage, crop production), (ii) perturbations to N transformation kinetics caused by acetylene inhibition of nitrification, and (iii) logistical constraints on the number of experimental variables which can be investigated concurrently.

Conclusions

This study supports the conclusion that a rye-vetch cover crop mixture may be superior to monoculture vetch or rye cover crops because of intermediate net N-mineralization rates resulting in decreased N leaching and denitrification losses, and better correspondence between N availability and crop N demands.

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